# INTERNATIONAL STANDARD 5518

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION•МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ•ORGANISATION INTERNATIONALE DE NORMALISATION

## Fruits, vegetables and derived products — Determination of benzoic acid content — Spectrophotometric method

*Fruits, légumes et produits dérivés — Détermination de la teneur en acide benzoïque — Méthode spectrophotométrique*

**First edition — 1978-05-01**

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ISO 5518:1978
https://standards.iteh.ai/catalog/standards/sist/274efe30-931e-4f27-a6f0-99477f2dbd32/iso-5518-1978

UDC 634.1/635 : 547.581.2 : 543.42 Ref. No. ISO 5518-1978 (E)

**Descriptors** : fruits, vegetables, fruit and vegetable products, chemical analysis, determination of content, benzoic acid, spectrophotometric analysis.



ISO 5518-1978 (E)

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International Standard ISO 5518 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in March 1977.

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# Fruits, vegetables and derived products — Determination of benzoic acid content — Spectrophotometric method

## 0 INTRODUCTION

The method for determining the benzoic acid content of fruits, vegetables and derivatives adopted by the Association of Official Analytical Chemists (AOAC), U.S.A., is based on the technique of peak emergence described by Stanley in 1959. The advantage of this method is that it is specifically intended for benzoic acid, with one exception : *p*-chlorobenzoic acid.

However, it is necessary to make a modification which consists in purifying the ethereal extract by chromic acid oxidation. This results in the elimination of the effect of colouring substances in certain vegetable products containing anthocyanins and, on the other hand, all the oxybenzoic acids and sorbic acid which may be present if several antiseptics have been used. Moreover, purification increases the sensitivity of the method.

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The improved technique is also more rapid.

As Stanley has already stated, the use of chloroform is not suitable for the determination of the benzoic acid content. Recent tests have stressed the difference existing between the partition coefficient for chloroform and water, equal to 5, and that for diethyl ether and water, equal to 33. The use of chloroform would require a greater number of washings and a dilution of the test solutions.

## 1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a method for determining the benzoic acid content of fruits, vegetables and derived products.

As chlorobenzoic acids are resistant to oxidation, the method cannot be applied in the presence of *p*-chlorobenzoic acid, as the absorption spectrum of this acid is close to that of benzoic acid. Neither may it be used in the presence of cinnamic acid, which is transformed into benzoic acid by chromic acid oxidation.

NOTE — The cinnamic acid determined as benzoic acid in this method exists generally only in the form of traces in vegetables, and therefore has no effect on the results obtained, except in the case of the cinnamon pod, which contains greater quantities.

## 2 PRINCIPLE

Homogenization of the product, followed by dilution and acidification of a test portion, extraction of the benzoic acid by diethyl ether, then alkaline re-extraction of this acid and purification by oxidation using acidified potassium dichromate. Determination by spectrophotometry of the purified benzoic acid dissolved in diethyl ether.

## 3 REAGENTS

All the reagents shall be of recognized analytical purity. The water used shall be distilled water or water of equivalent purity.

**3.1 Tartaric acid,** crystalline.

**3.2 Sodium hydroxide,** approximately 1 N solution.

**3.3 Potassium dichromate,** 33 to 34 g/l solution.

**3.4 Sulphuric acid** solution obtained by diluting 2 volumes of concentrated sulphuric acid ($\rho_{20}$ 1,84 g/ml) with 1 volume of water.

**3.5 Diethyl ether,** recently distilled.

**3.6 Benzoic acid,** 0,100 g/l standard solution in diethyl ether.

## 4 APPARATUS

Usual laboratory equipment, and in particular :

**4.1 Volumetric flasks,** capacity 50 ml, complying with ISO 1042.

**4.2 Beakers,** capacity 50 and 100 ml.

**4.3 Pipette,** capacity 20 ml, complying with ISO 648.

**4.4 Graduated pipettes,** complying with ISO/R 835.

**4.5 Flasks,** capacity 250 ml, having ground glass stoppers and made of borosilicate glass.

**4.6 Separating funnels,** capacity 500 ml.

**4.7 Water bath,** capable of being controlled at a temperature of 70 to 80 °C.

**4.8 Homogenizer.**

**4.9 Spectrophotometer** for determination in the ultra-violet range, equipped with a monochromator allowing measurement to the nearest 0,5 nm, with **silica cells** of optical path length 10 or 20 mm (preferably 20 mm so as to increase sensitivity) equipped with ground glass covers.

**4.10 Analytical balance.**

## 5 PROCEDURE

### 5.1 Preparation of the test sample

**5.1.1** *Liquid products* (juices, pulpy fluid products, syrups) *and thick products* (marmalades, jams)

Homogenize the laboratory sample after having carefully mixed it.

**5.1.2** *Solid products* (fruits, vegetables)

Cut a part of the laboratory sample into small pieces, remove seeds and carpellary cells, if necessary, and carefully homogenize approximately 40 g of the sample.

Frozen or deep-frozen products shall first be thawed in a closed container and the liquid formed during thawing shall be added to the product before homogenization.

### 5.2 Test portion

**5.2.1** *Liquid products*

Using a pipette (4.3), take 20 ml of the test sample (5.1), free from substances in suspension, dilute it with approximately 50 ml of water and transfer it into a 500 ml separating funnel (4.6) (separating funnel A).

NOTE — The test portion may also be taken by mass, by weighing, to the nearest 0,01 g, approximately 20 g of the test sample.

**5.2.2** *Pulpy fluid products*

Take 20 ml of the test sample (5.1). Place in a mortar and dilute with 20 ml of water. After decanting, filter the liquid.

Twice successively, take up the residue in 20 ml of water and filter after decanting.

Collect all the filtrates directly in a 500 ml separating funnel (4.6) (separating funnel A).

NOTE — The test portion may also be taken by mass, by weighing, to the nearest 0,01 g, approximately 20 g of the test sample.

**5.2.3** *Thick or solid products*

Weigh, to the nearest 0,01 g, approximately 10 g of the test sample (5.1) and, using 30 to 40 ml of water, transfer it into a 250 ml flask (4.5).

Add approximately 50 mg of sodium hydrogen carbonate (see note). Shake, then place on the water bath (4.7), controlled at 70 to 80 °C, and leave for 15 to 30 min. Filter the contents of the flask and rinse twice using 15 to 20 ml of water each time.

Collect all the filtrates in a 500 ml separating funnel (4.6) (separating funnel A). Allow to cool.

NOTE — The addition of sodium hydrogen carbonate is intended to neutralize the benzoic acid, of which traces could be lost by volatilization.

### 5.3 Extraction of the benzoic acid

**5.3.1** Introduce 1 g of the tartaric acid (3.1) into the separating funnel (A) containing the diluted test portion (5.2), add 60 ml of the diethyl ether (3.5) and shake carefully.

Allow to separate, then collect the ethereal layer in a second 500 ml separating funnel (4.6) (separating funnel B).

Wash the aqueous phase in the first separating funnel (A) with 60 ml of the diethyl ether.

Allow to separate, then collect the ethereal layer in the separating funnel (B) containing the first layer collected.

Proceed similarly with a third extraction with 30 ml of the diethyl ether and combine the ethereal layer with the first two in the separating funnel (B).

**5.3.2** Extract the benzoic acid from the ethereal solution by adding successively 10 ml and then 5 ml of the sodium hydroxide solution (3.2), and then twice 10 ml of water. After each addition, shake, then allow to separate and collect the aqueous phase.

Collect the aqueous phases in a dish. Place the dish on the water bath (4.7), controlled at 70 to 80 °C, and leave until the volume of the alkaline solution is reduced by approximately half, to remove the residual dissolved diethyl ether.

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### 5.4 Purification of the benzoic acid

After cooling, pour the contents of the dish into a 250 ml flask (4.5) containing a mixture of 20 ml of the sulphuric acid solution (3.4) and 20 ml of the potassium dichromate solution (3.3). Stopper the flask, shake and leave for at least 1 h.

NOTES

1 Other preservatives derived from benzoic acid may be present. In this case, leave the flask for at least 3 h, to oxidize completely the three hydroxybenzoic acids and prevent any interference in the determination. The extension of the reaction time creates no problem as the benzoic acid resists this oxidizing mixture.

2 When the initial product also contains sorbic acid, it is necessary to prolong oxidation for 24 h so as to ensure the complete destruction of this acid.

### 5.5 Extraction of the purified benzoic acid

Extract the benzoic acid by treating the above solution (5.4) twice with 20 to 25 ml of the diethyl ether, collecting the ethereal solutions. Wash the ethereal solutions twice with several millilitres of water. After decanting very carefully, filter through a dry filter paper and collect the filtrate in a 50 ml volumetric flask (4.1). Then wash the filter with several millilitres of the diethyl ether, adding sufficient washing solvent to the filtrate to dilute to the mark.

### 5.6 Determination

Using the spectrophotometer (4.9), measure the absorbance of the ethereal solution (5.5) in relation to the absorbance of the pure diethyl ether at 267,5 to 272 nm and 276,5 nm (see note).

The absorbance due to benzoic acid is given by the formula for the differential measure of emergence at 272 nm :

$$A_2 - \frac{A_1 + A_3}{2}$$

where

$A_1$ is the absorbance at 267,5 nm;

$A_2$ is the absorbance at 272 nm;

$A_3$ is the absorbance at 276,5 nm.

NOTE — The examination of the absorption spectrum of the ethereal solution of purified benzoic acid allows the characterization of this product by the presence of two peaks at 272 and 279 nm.

The benzoic acid extracted by the diethyl ether is determined by the measurement of the relative height of the peak at 272 nm with respect to the straight line which joins the points on the abscissa between 267,5 and 276,5 nm.

### 5.7 Number of determinations

Carry out two determinations on the same test sample (5.1).

### 5.8 Plotting the calibration curve

Into a series of six 50 ml volumetric flasks (4.1), introduce respectively 5 — 7,5 — 10 — 12,5 — 15 — 20 ml of the standard benzoic acid solution (3.6). Dilute to the mark with the diethyl ether (3.5).

The solutions obtained contain respectively 10 — 15 — 20 — 25 — 30 — 40 mg of benzoic acid per litre.

Carry out differential measurement of these solutions by proceeding as described in 5.6.

Plot the curve showing the differential measurements with respect to the number of milligrams of benzoic acid per litre indicated above.

## 6 EXPRESSION OF RESULTS

### 6.1 Method of calculation and formulae

#### 6.1.1 *Test portion taken by pipetting*

The benzoic acid content, expressed in milligrams per litre of product, is given by the formula

$$m_2 \times \frac{50}{20} = 2{,}5\, m_2$$

where $m_2$ is the mass, in milligrams, of benzoic acid read on the calibration curve (5.8).

#### 6.1.2 *Test portion taken by weighing*

The benzoic acid content, expressed in milligrams per kilogram of product, is given by the formula

$$m_2 \times \frac{50}{m_1}$$

where

$m_1$ is the mass, in grams, of the test portion (5.2);

$m_2$ is the mass, in milligrams, of benzoic acid read on the calibration curve (5.8).

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### 6.2 Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst shall not exceed 10 mg of benzoic acid per litre or per kilogram, depending on the individual circumstances.

NOTE — The method allows the determination of the quantity of benzoic acid to the nearest 2 mg when the product contains less than 50 mg per litre or per kilogram.

## 7 TEST REPORT

The test report shall show the method used and the result obtained. It shall also mention all operational details not specified in this International Standard, or regarded as optional, as well as any incidents likely to have affected the result.

The report shall give all information necessary for the complete identification of the sample.

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